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Retranslocation of boron in broccoli and lupin during early reproductive growth

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The objective of the present study was to determine if boron (B) retranslocation depends on plant-B status and external-B supply. The stable ^{10}B isotope was supplied to the root system of broccoli (*Brassica oleracea* var. *italica* Plenck cv. Commander) and lupin (*Lupinus albus* L. cv. Ultra) plants to provide a quantitative picture of B distribution during early reproductive development. Regardless of the B regime (i.e. continuous supply with luxury, sufficient or deficient B; transfer at inflorescence emergence from either a luxury- or sufficient-B supply to a deficient one) and whether ^{10}B was acquired before or during inflorescence development, a significant proportion of the B recovered in broccoli florets and lupin fruit was ^{10}B enriched. B acquired during inflorescence development was an important source of B for reproductive structures, but the relative importance of B acquired before and after inflorescence emergence appeared to be species dependent. The occurrence of B retranslocation was not dependent upon the induction of B deficiency. The concentrations of B in phloem exudates (0.38 to 0.03 m*M*) were 4- to 23-fold those in xylem sap, and more similar to the concentrations in the reproductive structures (0.86 to 0.07 m*M*) than those in source leaves (2.4 to 0.19 m*M*). The decreasing acropetal gradient of tissue-B concentrations with luxury-B supply declined dramatically or was reversed in plants grown with sufficient or deficient B. The data are consistent with B being a phloem-mobile element, and suggest that newly acquired B is particularly important during the early reproductive growth of plants.

Key words – Boron, *Brassica oleracea*, broccoli, lupin, *Lupinus albus*, mobility, phloem, retranslocation, xylem.

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Introduction

Different nutrient elements have different mobilities in plants (Pate 1975, Welch 1986). This differential mobility is related to the relative importance of xylem vs phloem in providing nutrients to developing sinks. The first route involves the primary translocation of minerals by the water stream in the xylem, whereas the second route is secondary translocation or retranslocation in the phloem, away from sites of initial deposition to sites that do not lose water readily. Macronutrients, with the exception of calcium, are readily retranslocated. In contrast, the retranslocation of micronutrients may depend on a number of factors, including stage of growth, plant nutrient status and external supply of the nutrient.

The distribution of B in plants is often related to its translocation in the xylem (Eaton 1944, Raven 1980, Oertli 1993), and there is considerable controversy regarding the role that phloem plays in providing B to developing sinks (Shelp et al. 1995). Growing evidence from different plant species suggests that B distribution is altered in response to continuous B starvation or to removal of B after a period of adequate supply (see references in Shelp et al. 1995). Examples include decreased

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B content in mature leaves of grape (Scott and Schrader 1947), broccoli (Benson et al. 1961, Shelp et al. 1992a, Liu et al. 1993), cotton and turnip (McIlrath 1965), and unchanging B content in fruits of peanut and subterranean clover (Campbell et al. 1975) when the B supply to plants is interrupted. In both greenhouse (Shelp et al. 1992b) and field-grown broccoli (Liu et al. 1993) the typical B gradient, where concentration decreases from old to young plant parts, disappears in plants grown with a B supply that is inadequate for maximum floret yield.

These studies, which are based on net changes in contents or concentrations of B, indicate that B is retranslocated under conditions of B deficiency, but they do not indicate if B retranslocation depends on plant-B status and external-B supply, and they do not distinguish between previously and newly acquired sources of B. Determination of retranslocation as a function of plant-B status and external-B supply requires the use of isotopes. The stable isotopes ^{10}B and ^{11}B have been used to monitor the short-distance transport of B (Thellier et al. 1979, Chamel et al. 1981, Jimenez et al. 1988) and the movement of foliar-applied B (Hanson 1991, Brown et al. 1992, Shu et al. 1993, 1994, Pichioni et al. 1995, Shelp et al. 1996); however, they have not been used to study the retranslocation of B supplied to the root system.

The present study determined if ^{10}B acquired via the root system either before or after the onset of reproductive growth is retranslocated to reproductive structures of broccoli (i.e. inflorescence) and lupin (i.e. primary fruit-bearing inflorescence) plants grown with various B regimes.

Abbreviations – DAIE, days after inflorescence emergence; DM, dry matter; Lvs, leaves; RP, relative partitioning; RSA, relative specific allocation.

Materials and methods

Plant material

Broccoli (*Brassica oleracea* var. *italica* Plenck cv. Commander) and lupin (*Lupinus albus* L. cv. Ultra) plants were grown under greenhouse conditions essentially as previously described (Shelp et al. 1992a). Broccoli seeds were germinated in seedling trays filled with vermiculite and supplied with one-quarter-strength nutrient solution (see below) not containing B (prepared with reverse-osmosis water and plastic containers to minimize B contamination). After 3 weeks, individual seedlings were transferred to 6-l pots containing vermiculite and fertilized every second day with 2 l of a modified Hoagland's solution (pH 6.5) (Hoagland and Arnon 1950) containing in mg l^{-1}: Ca, 60; K, 312; Mg, 49; N, 266 (161 as NO_3^- and 105 as NH_4^+); P, 77; S, 128; Cl, 0.1; Mn, 0.1; Zn, 0.05; Cu, 0.02; Mo, 0.02; Co, 0.01 and Fe, 1.1 (as Fe-EDTA). Lupin seeds were directly sown into 1-l pots filled with vermiculite and fertilized daily with nutrient solution containing in mg l^{-1}: Ca, 120; K, 127; Mg, 12; N (as NO_3^-), 112; P, 15.5; S, 24.1; Cl, 0.1; Mn, 0.02; Zn, 0.01; Cu, 0.005; Mo, 0.004; Co, 0.003 and Fe, 2.2 (as EDTA). After 3 weeks, the concentrations of N, K and Ca in the nutrient solution were doubled. The plants were maintained under average day/night temperatures of 25/18°C and a 16-h light period. Natural lighting was supplemented by high-intensity sodium vapour lamps (model SDN AGRO 430 W 213, Philips Lighting Co., Somerset, NJ, USA) yielding a quantum flux density at pot level of 100 μmol m^{-2} s^{-1}.

Experimental design

Experiment one was carried out during May–July, 1991. Three B treatments (natural abundance of ^{10}B is 19.098%, International Union of Pure and Applied Chemistry 1991) (2.5, 0.5 and 0.05, and 1.2, 0.3 and 0.03 mg l^{-1} for broccoli and lupin, respectively) were provided up to inflorescence emergence (70 days for broccoli and 60 days for lupin). For the next 14 or 21 days, experimental plants were supplied with 95.5% ^{10}B-enriched boric acid (Cambridge Isotope Laboratories, Woburn, MA, USA) at either the same concentration supplied previously or at the 0.05 mg B l^{-1} concentration. At the beginning of the ^{10}B-feeding period, all pots were thoroughly washed with reverse-osmosis water. Control plants were supplied continuously throughout development with the same concentration of natural abundance B. All treatments were allocated in a complete randomized block design with five replicates for each harvest time. Broccoli plants were harvested at 0, 4, 8 and 14 days after inflorescence emergence (DAIE), and divided into the following strata: leaves (Lvs) 1–4, 5–8, 9–14, 15–22, stem+petioles (including midribs), and florets. Leaves that dropped from the lowest stratum for each species before final harvest were collected. Lupin plants were harvested at 0, 7, 14 and 21 DAIE, and divided into the following strata: Lvs 1–8, 9–16 on the primary stem, primary stem+petioles, primary inflorescence, secondary stem+petioles, Lvs 17–28 on the secondary stem, and secondary inflorescence. All tissues were oven-dried to a constant dry matter (DM) at 60°C and analyzed for B as described below. For broccoli at 4 DAIE, insufficient floret tissue was available for B analysis.

In experiment two, which was carried out during May–July, 1993, control broccoli and lupin plants were supplied throughout development with 99.9% ^{11}B-enriched boric acid (Eagle Picher Industries, Quapaw, OK, USA) at concentrations of 0.5 (broccoli) and 0.3 mg l^{-1} (lupin), respectively. For experimental plants, the ^{11}B-enriched boric acid was replaced with ^{10}B-enriched boric acid at equivalent molar concentrations for the 10- and 15-day periods just before inflorescence emergence in broccoli and lupin, respectively; after inflorescence emergence, ^{11}B was supplied again. At both the beginning and end of the ^{10}B-feeding period, all pots were thoroughly washed with reverse-osmosis water. Plants were allocated in a complete randomized block design with five replicates for each harvest time. Broccoli plants were harvested at 0, 3, 6, 9, 12 and 15 DAIE, and lupin plants at 0, 5, 10, 15, 20, 25 and 30 DAIE; they were divided into strata as described above and dried to a constant weight at 60°C.

Collection of phloem exudate and xylem sap

Phloem exudate and xylem sap were obtained from each plant harvested over the entire time course. Plants were fertilized at 0900 h, about 3 h after the beginning of the photoperiod. Two hours later, phloem exudate was collected as droplets, using an automatic pipetter equipped with plastic tips, from shallow incisions in the stems of the attached broccoli inflorescence (Shelp 1987), from shallow incisions in the lupin stem two nodes below the base of the primary inflorescence, and from excised tips of lupin fruit (Pate and Sharkey 1974); fruit tips were excised twice in order to increase the exudate volume for analysis. Immediately after collection of phloem exudate, the plant was harvested at the vermiculite surface and xylem sap collected for 1 h from the root stump; sap collected during the first 5 min was discarded. Prior to the preparation of analyte solutions for isotope analysis, the acidity of the translocation fluids was measured using narrow-range pH paper.

Total boron and isotope ratio analyses

Dried plant tissues were digested overnight with HNO_3 in closed vessels as described by Topper and Kotuby-Amacher (1990), and aliquots of the translocation fluids were diluted with 1% HNO_3. Isotope ratios and content measurements were determined for ^{10}B and ^{11}B using a PlasmaQuad 2+/Turbo inductively coupled plasma mass spectrometer (VG Elemental, Winsford, Cheshire, UK) with previously reported operating conditions (Vanderpool et al. 1994). Each sample was spiked with beryllium (Esar, ICP standard solution, 1000 ppm Be, Specpure) as an internal standard and data were normalized to the beryllium signal.

Samples were run concurrently with 75 ppm standards prepared from National Institute of Science and Technology (NIST) Standard Reference Material (SRM) number 951, boric acid and assay standard, and used for instrument bias corrections (Catanzaro et al. 1970). Isotope ratios, R = $^{11}B^{10}B$, were bias corrected by

$$\mathrm{Smp}_{R_{corr}} = \mathrm{Smp}_{R_{obs}} \times \mathrm{SRM}_{R_{lit}} / \mathrm{SRM}_{R_{obs}}$$

where: $\mathrm{Smp}_{R_{corr}}$ is the corrected sample ratio; $\mathrm{Smp}_{R_{obs}}$ is the observed sample ratio; $\mathrm{SRM}_{R_{lit}}$ is the SRM literature ratio of $R(^{11}B/^{10}B) = 4.04362 \pm 0.00137$ (Vanderpool and Johnson 1992); and $\mathrm{SRM}_{R_{obs}}$ is the SRM observed ratio.

Retranslocation parameters

^{10}Boron isotope contents are expressed as ^{10}B enrichment which is the difference between treated and control plants. The atom percent ^{10}B of control plants grown with natural abundance B did not substantially change over the period of inflorescence development studied (e.g. the broccoli inflorescence decreased from 20.70 ± 0.14 to 20.00 ± 0.09 over 14 days). To minimize complications due to B exchange between the vermiculite (Sims and Bingham 1968) and nutrient solution, and within the root system, retranslocation parameters for the shoot were calculated as a function of the average isotope composition of the xylem sap obtained from independent samplings up to and including the harvest time of interest; this value for ^{10}B enrichment of xylem sap therefore reflects the changing nature of the B supply to the shoot.

Relative specific allocation (RSA) is the proportion of B atoms that are enriched in ^{10}B in a stratum or in the shoot relative to the xylem sap (determined from the absolute amounts of ^{11}B and ^{10}B) and was calculated using the following equation modified from Cliquet et al. (1990).

$$\mathrm{RSA} = \frac{\text{atom\% } ^{10}\text{B enrichment sample}}{\text{atom\% } ^{10}\text{B enrichment xylem sap}} \times 100$$

Relative partitioning (RP) expresses the distribution among shoot strata of the B atoms that are enriched in ^{10}B and was calculated using the following equation modified from Cliquet et al. (1990).

$$\mathrm{RP} = \frac{(\text{RSA stratum}) \times (\text{DM stratum})}{(\text{RSA shoot}) \times (\text{DM shoot})} \times \frac{(\text{B concentration stratum})}{(\text{B concentration shoot})} \times 100$$

Results

Translocation of boron acquired during inflorescence development

In experiment one, broccoli plants were grown under five different B regimes over the growing period. Three regimes consisted of a continuous supply of B at 2.5, 0.5 or 0.05 mg l^{-1} nutrient solution both before and after inflorescence emergence. In the other two, B was supplied at either 2.5 or 0.5 mg l^{-1} up to inflorescence emergence, then at 0.05 mg l^{-1} after inflorescence emergence. At 14 DAIE, the plants receiving 0.05 mg B l^{-1} had a 30% lower dry matter content than those receiving either 2.5 or 0.5 mg B l^{-1} (Tab. 1), and exhibited signs of internal hollowing, stem corkiness and midrib cracking. The B concentrations of florets varied about 3-fold, and those of Lvs 1–4 and 9–14 varied about 7- and 12-fold, respectively, over the 5-fold range in B supply.

The xylem sap of broccoli was mildly acidic (pH 5.5–6.0), whereas the phloem exudate was near neutral (pH 7.2–7.3) confirming the origin of these fluids in broccoli (Shelp 1987). Boron was present in xylem saps and phloem exudates (replicates were pooled for analysis) at mean (± SE) concentrations ranging from 0.77 ± 0.05 to 0.14 ± 0.01 µg B ml^{-1} and from 3.33 to 0.31 µg B ml^{-1}, respectively. The ^{10}B enrichment of xylem saps, which ranged from 6.3 to 60.2 atom% excess, was linearly related to the B supply during inflorescence devel-

Tab. 1. Dry matter content of broccoli plants and tissue-B concentrations of some strata at 14 DAIE. Arrows indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5).

Regime (mg B l^{-1})	Plant DM (g)	Tissue-B concentration of stratum ($\mu g\ g^{-1}$ DM)		
		Lvs 1–4	Lvs 9–14	Florets
2.5→2.5	174 ± 19	134 ± 8	181± 9	64 ± 3
2.5→0.05	167 ± 18	108 ± 6	97 ± 5	56 ± 6
0.5→0.5	158 ± 15	37 ± 5	29 ± 2	56 ± 7
0.5→0.05	149 ± 13	29 ± 2	22 ± 2	28 ± 4
0.05→0.05	121 ± 11	19 ± 1	15 ± 2	21 ± 3

opment (Tab. 2). To calculate RSA and RP for each harvest, the average of the ^{10}B enrichments found in the preceding harvests, as well as the one of interest, was used; for example, the average ^{10}B enrichment of plants receiving 2.5 mg B l^{-1} for 14 days was 52.3 atom% ^{10}B excess.

When B was supplied continuously at the same concentration, RSAs progressively increased from the bottom to the top of the broccoli plant (Fig. 1). Shifting the B treatment from 2.5 to 0.05 mg l^{-1} reduced the magnitude of RSAs for all strata and prevented the increases in RSA found with 2.5 mg B l^{-1} during the interval 8–14 DAIE. Similar trends were observed for the shift from 0.5 to 0.05 mg B l^{-1}, but the magnitude of RSAs was reduced by a smaller degree. With B supplied continuously at 0.05 mg l^{-1}, the magnitude of the RSAs for all strata except the florets was usually higher by 8 DAIE than the corresponding RSA from the other B regimes. Furthermore, these high RSAs were transient. The RSAs of florets at 14 DAIE ranged from 68 to 99%.

When B was supplied continuously at the same concentration, RPs increased to a maximum in all strata, except in florets by either 4 or 8 DAIE (Fig. 2). This was followed by declines in all strata except in florets where RPs increased. With a continuous supply of B at 2.5 mg l^{-1}, the highest RP was found in Lvs 9–14, whereas with 0.5 and 0.05 mg B l^{-1}, it was found in stem+petioles. The RP patterns were similar between 2.5 mg l^{-1} and a shift

Tab. 2. Time course of ^{10}B enrichment (atom% ^{10}B excess) of xylem sap from broccoli plants fed with ^{10}B-enriched boric acid. Arrows indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5).

Regime (mg B l^{-1})	Days after inflorescence emergence		
	4	8	14
2.5→2.5	47.7 ± 0.5	48.9 ± 0.05	60.2 ± 0.3
2.5→0.05	6.3 ± 0.1	9.8 ± 0.1	10.2 ± 0.3
0.5→0.5	39.9 ± 1.0	45.6 ± 0.8	40.3 ± 2.7
0.5→0.05	9.6 ± 0.1	11.8 ± 0.1	9.5 ± 0.1
0.05→0.05	9.4 ± 0.2	48.9 ± 0.05	10.2 ± 1.7

from 2.5 to 0.05 mg B l^{-1}, and between 0.5 mg B l^{-1} and a shift from 0.5 to 0.05 mg B l^{-1}. At 14 DAIE, the RPs of florets ranged from 44 to 79%.

Lupin plants were grown with a continuous supply of B at 1.2, 0.3 or 0.03 mg l^{-1} nutrient solution. At 21 DAIE the dry matter content of plants receiving 0.03 mg B l^{-1} was only 60% of that of plants receiving 1.2 or 0.3 mg B l^{-1} (Tab. 3); fruit yield was only 5% (data not shown). Apart from the reduction in yield, no external symptoms of B deficiency were evident. The B concentrations of the primary inflorescence and mature leaves (Lvs 1–8, 9–16), respectively, varied about 3- and 4- to 7-fold with the 40-fold range in B supply.

The xylem sap of lupin was mildly acidic (pH 5.5–6.0), whereas the phloem exudate collected from various locations on the plant were near neutral (pH 7.2–7.9) confirming the origin of these fluids in lupin (Pate and Sharkey 1974). Boron was present in xylem saps and phloem exudates at mean (± SE) concentrations ranging from 1.25 ± 0.03 to 0.13 ± 0.04 and from 4.21 ± 0.15 to 2.98 ± 0.31 $\mu g\ ml^{-1}$, respectively. The ^{10}B enrichment of xylem saps, which ranged from 0.6 to 27.8 atom% excess, was linearly related to the B supply during inflorescence development (Tab. 4).

Leaves 1–8, 9–16, primary stems and petioles, and primary inflorescences exhibited maximum RSAs at 7 DAIE (Fig. 3); for the primary inflorescence these ranged from 55 to 100%. Maximum RSAs in the remaining strata were found at 14 to 21 days. For each B regime, the maximum RSAs of some strata (e.g. primary inflorescence in the inadequate-B regime) was followed by declines. The final RSAs of the primary and secondary inflorescences ranged from 9 to 69% and 15 to 70%, respectively. Leaves 1–8, 9–16 and primary stem+petioles of plants receiving 0.3 mg B l^{-1} exhibited maximum RPs at 7 DAIE, followed by declines (Fig. 4). These declines were accompanied by increases in the RPs of both secondary stem+petioles and Lvs 17–28, and final RPs of 10 and 4% for primary and secondary inflorescences, respectively. The other two regimes (1.2 and 0.03 mg B l^{-1}) had similar trends and RP values (data not shown).

Retranslocation of B acquired before inflorescence development

In experiment two, broccoli plants were grown continuously with 0.5 mg B l^{-1}. During inflorescence development, marked increases in the DM of florets and stem+petioles were observed (data not shown). Only slight increases were found in other strata. At 15 DAIE, the mean (± SE) total DM was 106.5 ± 11.3 g. Leaves 1–4, 9–14 and florets had mean (± SE) tissue-B concentrations of 194.2 ± 4.0, 155.6 ± 4.5 and 38.3 ± 1.0 $\mu g\ g^{-1}$ DM, respectively. The xylem sap was mildly acidic (pH 5.5–6.0), whereas the phloem exudate was near neutral (pH 7.2–7.3). Boron was present in xylem sap and phloem exudate at mean (± SE) concentrations of 0.19 ± 0.02 and 3.43 ± 0.11 $\mu g\ ml^{-1}$, respectively. Over the time course the

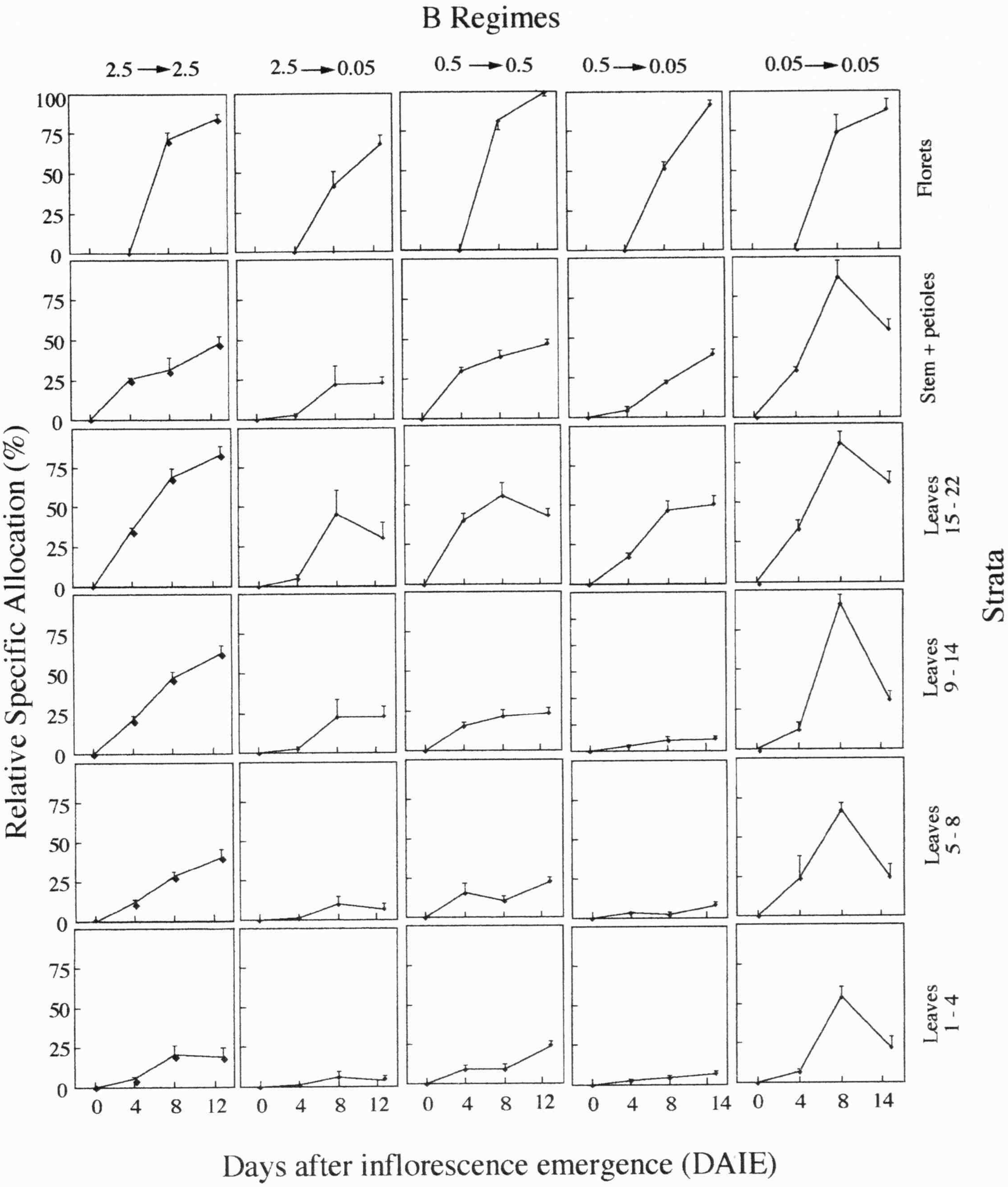


Fig. 1. Relative specific allocation (% of B that was enriched) during broccoli-inflorescence development of B acquired after inflorescence emergence. Arrows in the upper legend indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5); where the SE is not evident, it is within the symbol.

^{10}B enrichment of xylem sap declined from 33.5 ± 1.6 to 2.5 ± 0.4 atom% ^{10}B excess (data not shown).

Boron acquired in the 10-day period just before inflorescence emergence accounted for less than 1.2% of the total B in each broccoli stratum (data not shown). At 0 DAIE, the RPs of all strata except florets ranged from about 3 to 50%; most of these did not change (e.g. Lvs 1–4) or declined slightly (e.g. stem+petioles and Lvs 9–14) with development (Fig. 5). In contrast, the RPs of florets generally increased, up to a maximum of about 8% at 15 DAIE.

Lupin plants were grown continuously with 0.3 mg B l^{-1}. During the 30-day period of inflorescence development, DM accumulated in primary and secondary stem+petioles, primary inflorescence and secondary Lvs 17–28; Lvs 1–8 were unchanged, whereas Lvs 9–16 and the secondary inflorescence declined after 20 DAIE (data not shown). At about 10 DAIE, 5–7 fruits were present on the primary inflorescence. At 30 DAIE, the mean (± SE) total DM of the plant was 14.9 ± 1.2 g, and Lvs 1–8, 9–16 and primary inflorescence had mean (± SE) tissue-B concentrations of 32.8 ± 0.8, 27.9 ± 2.1 and 12.1 ± 0.6 µg

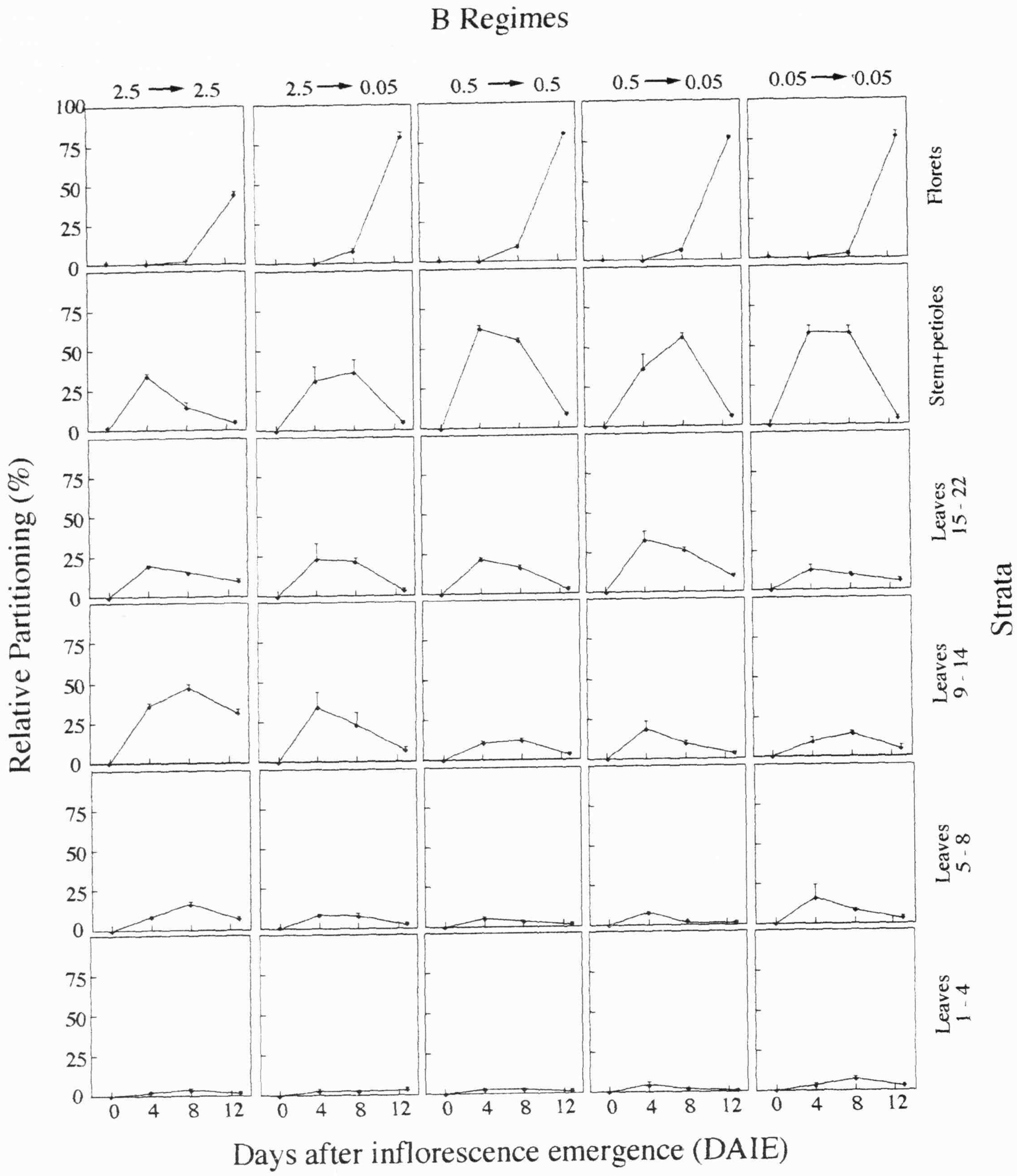


Fig. 2. Relative partitioning (% of total B enrichment) during broccoli-inflorescence development of B acquired after inflorescence emergence. Arrows in the upper legend indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5); where the SE is not evident, it is within the symbol.

g^{-1} DM, respectively. The xylem sap was slightly acidic (pH 5.9–6.7), and the phloem exudate slightly alkaline (pH 7.7–8.0). Boron was present in xylem sap and phloem exudate at mean (± SE) concentrations of 1.50 ± 0.07 and 4.08 ± 0.17 μg ml^{-1}, respectively. Over the time course, the ^{10}B enrichment of xylem sap declined from 28.6 ± 1.8 to 19.9 ± 2.5 atom% ^{10}B excess.

Boron acquired during the 15-day period just before emergence of the primary inflorescence accounted for less than 2% of the total B in each lupin stratum (data not shown). The RPs ranged from 20–50% at 0 DAIE (Fig. 5). Subsequently, these declined markedly with time as more of the B acquired just before inflorescence emergence was partitioned into new strata, particularly Lvs 17–28 and the primary inflorescence. At 30 DAIE, approximately 21% of the ^{10}B was recovered in the primary inflorescence.

Discussion

In the present study, the term retranslocation describes the transfer of solutes from xylem to phloem; this exchange process may take place in stem and leaf veins (i.e. direct) or via the leaf mesophyll (i.e. indirect) (Pate 1975, DaSilva and Shelp 1990). During indirect transfer, the solutes may be rapidly exported or stored and ex-

Tab. 3. Dry matter content of lupin plants and tissue-B concentrations of some strata at 21 DAIE. Arrows indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescenc emergence. Data represent the mean ± SE (n = 5).

Regime (mg B l^{-1})	Plant DM (g)	Tissue-B concentration of stratum (µg g^{-1} DM)		
		Lvs 1–8	Lvs 9–16	Primary inflorescence
1.2→1.2	11.2 ± 0.3	58.8 ± 3.8	63.9 ± 2.0	20.8 ± 2.8
0.3→0.3	11.9 ± 0.5	24.0 ± 2.6	25.0 ± 1.7	14.6 ± 1.4
0.03→0.03	6.9 ± 0.4	14.4 ± 0.6	8.9 ± 0.8	5.6 ± 0.4

Tab. 4. Time course of ^{10}B enrichment (atom% ^{10}B excess) of xylem sap from lupin plants fed with ^{10}B-enriched boric acid. Arrows indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5).

Regime (mg B l^{-1})	Days after inflorescence emergence		
	7	14	21
1.2→1.2	9.9 ± 0.3	13.2 ± 0.5	27.8 ± 0.7
0.3→0.3	8.2 ± 0.7	9.4 ± 0.6	20.6 ± 0.5
0.03→0.03	0.6 ± 0.1	0.6 ± 0.1	4.6 ± 0.5

ported at a latter stage of development (usually denoted by the term mobilization). It has been common to study the mobilization of nutrient elements, and in particular B, by determining the time course of net changes in contents or concentrations in various plant parts often in response to induced nutrient deficiency (see references in Introduction). However, such data are complicated by the simultaneous import and export of nutrient elements, and provide little information on retranslocation in plants given a nondeficient supply of nutrient.

With the recent introduction of commercially available inductively coupled-plasma mass spectrometers, it is now possible to use enriched mass isotopes of B to trace the dynamics of B translocation in plants as a function of plant-B status and external-B supply. In the present study, enriched ^{10}B was supplied to the root sys-

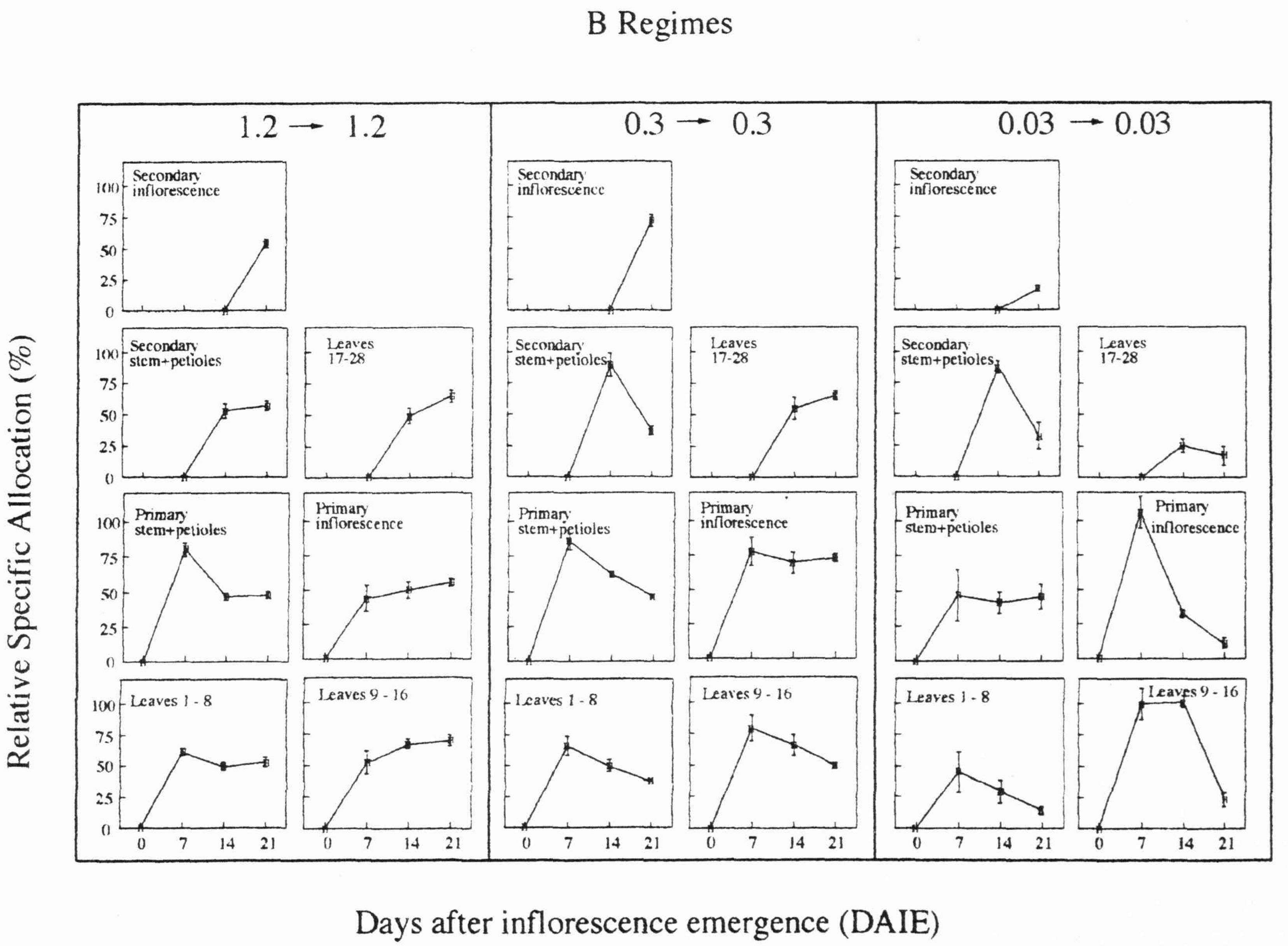


Fig. 3. Relative specific allocation (% of B that was enriched) during lupin-inflorescence development of B acquired after inflorescence emergence. Arrows in the upper legend indicate a shift from various B concentrations supplied at natural abundance to those enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5); where the SE is not evident, it is within the symbol.

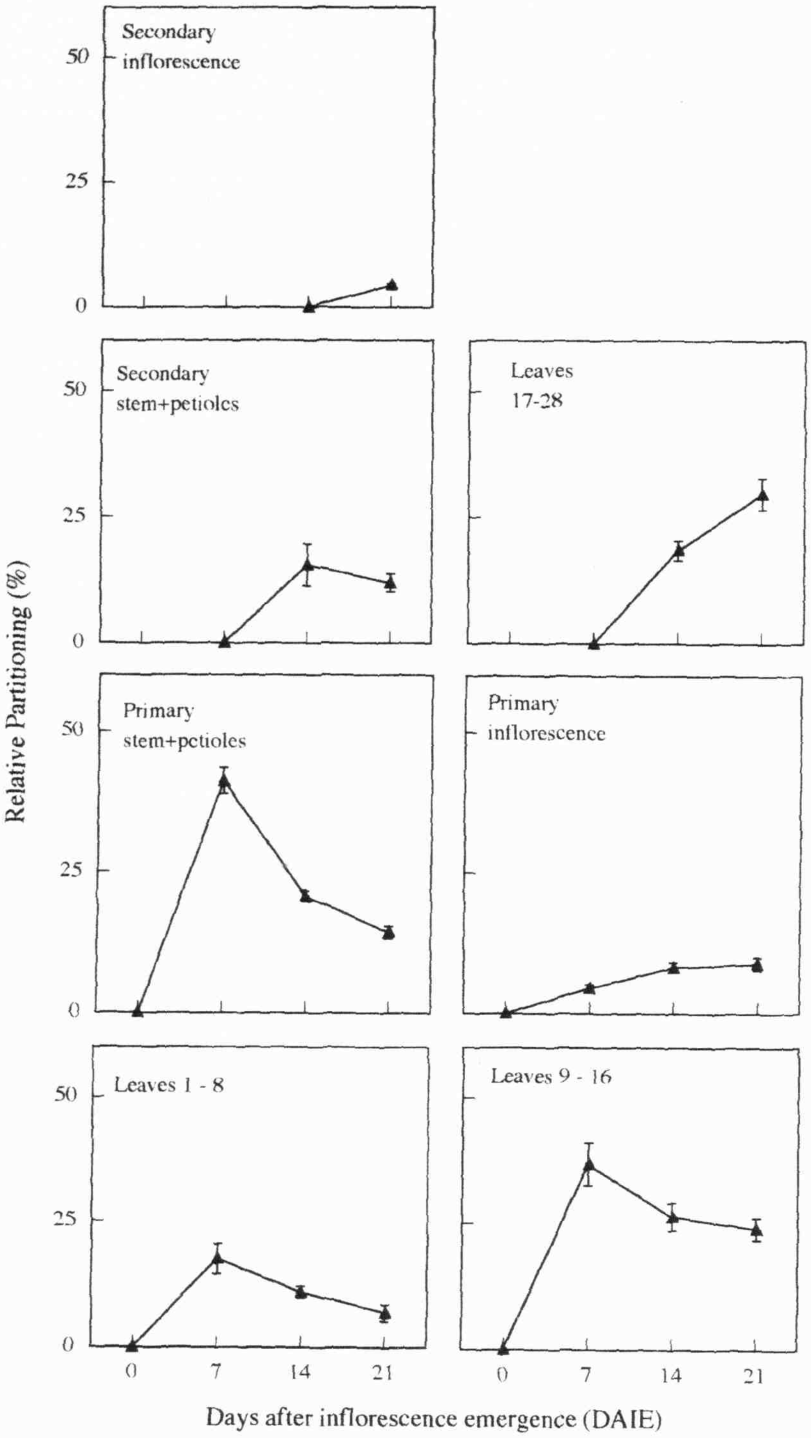


Fig. 4. Relative partitioning (% of total B enrichment) during lupin primary-inflorescence development of B acquired after inflorescence emergence. Plants were supplied initially with 0.3 mg B l^{-1} at natural abundance, then shifted to 0.3 mg B l^{-1} enriched in ^{10}B at inflorescence emergence. Data represent the mean ± SE (n = 5); where the SE is not evident, it is within the symbol.

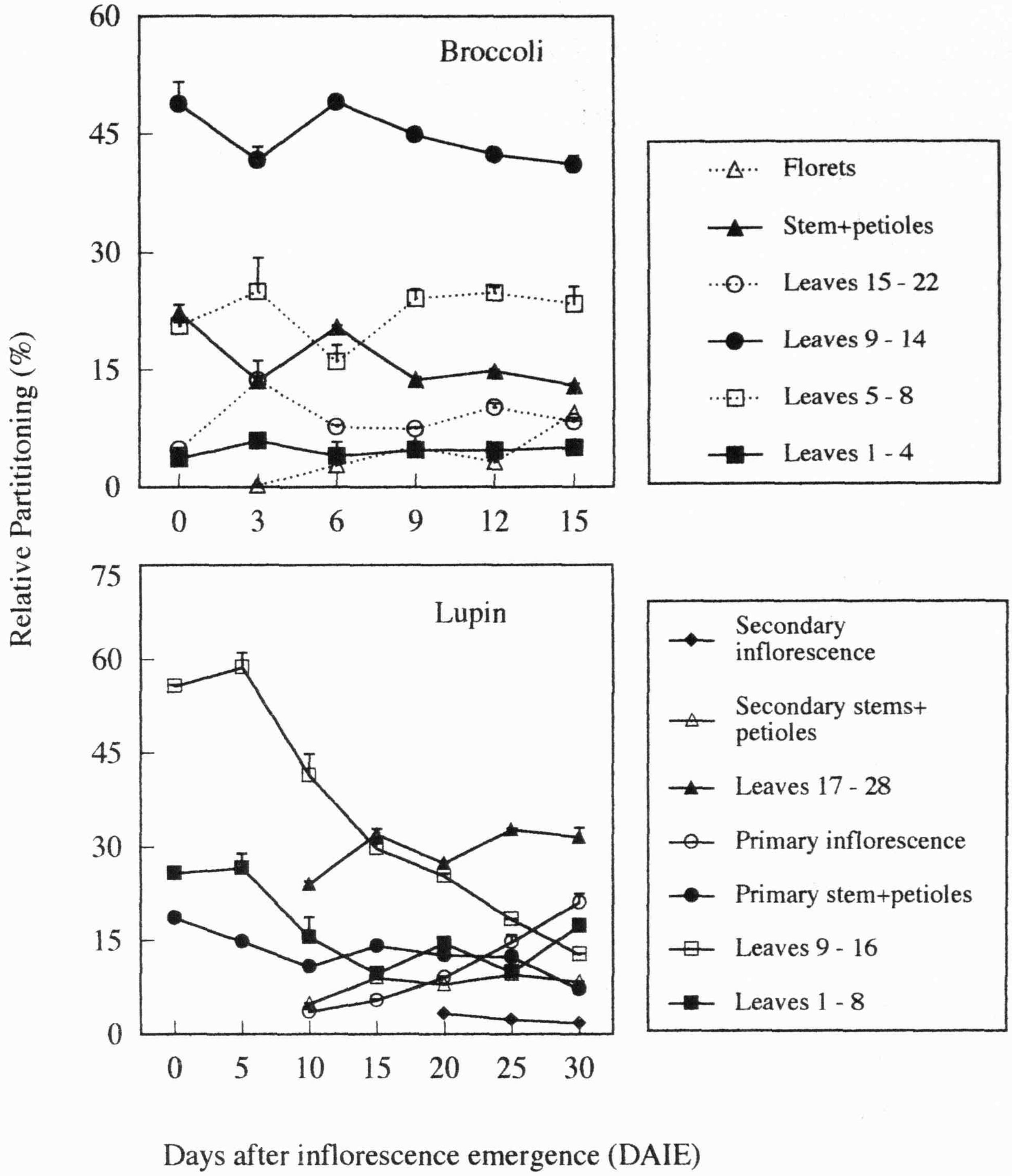
tem over an extended period, thereby providing a quantitative picture of the distribution of B acquired before or after inflorescence emergence. As it is our desire to adopt this method for determining the effectiveness of fertilizer-B application on shoot yield under conditions where native B is also present (i.e. field conditions), it was important to minimize complications due to B exchange between the rooting medium (e.g. vermiculite) and nutrient solutions, and within the root system. Therefore, the root system was not recovered and the retranslocation parameters for B were expressed as a function of the isotopic composition of xylem sap.

In the present study, both broccoli and lupin plants were provided with a range of external-B supply. With a continuous supply of the lowest B concentration, the final yields of plant and reproductive structures were reduced, and typical signs of hollow stem were seen in broccoli (Shelp et al. 1992a). With the other two B concentrations, the yields were higher but similar to each other. However, the tissue-B concentrations increased with the B supply. Thus, the levels of B supply chosen were considered as luxury, sufficient and deficient.

Plants grown with luxury-B supply exhibited a decreasing acropetal gradient of tissue-B concentrations. While this trend has been interpreted as evidence against B retranslocation, the gradient declined dramatically or was reversed in plants grown with sufficient or deficient B. These data are consistent with those reported previously for a number of plant species, including broccoli (see Shelp 1995). If B is mobile only in xylem, tissue-B should always be highest in source leaves. The B concentrations ranged from 2.4 to 0.19 m*M* in source leaves (assuming 15% DM as in Shelp 1988), from 0.86 to 0.07 m*M* in florets and fruits, and from 0.38 to 0.03 m*M* in phloem exudates. Previously, Shelp et al. (1992a) provided evidence showing that broccoli plants supplied with sufficient B have source leaves, florets, and phloem exudate with B concentrations of 4.6, 0.4 and 0.3 m*M*, respectively (see Shelp 1995). Tammes and van Die (1966) reported that in *Yucca flaccida* the B concentrations in source leaves, inflorescence and phloem exudate are 1.0, 0.3 and 0.2 m*M*. Thus, the phloem exudate has B concentrations that are more similar to those in reproductive structures than in source leaves. Furthermore, the B concentrations in phloem were 4- to 23-fold those in xylem sap collected as root bleeding sap. It is noteworthy that the B concentration in xylem sap of intact broccoli and lupin plants receiving sufficient B, estimated from data on shoot-B accumulation and water usage, was about 5 μM (A. J. Kitheka and B. J. Shelp, unpublished data). These phloem:xylem concentration ratios for B are similar to those for phloem-mobile elements such as nitrogen, phosphorus and potassium, but are considerably higher than those for phloem-immobile elements or nutrients such as calcium and nitrate (Pate 1975, Shelp 1987). Together, these results indicate that phloem, rather than xylem, is the predominant source of B for developing sinks and are consistent with B being a phloem-mobile element.

In the present study, we investigated the retranslocation of soil-derived B to plant parts that do not readily transpire and are predominantly phloem fed, broccoli florets and lupin inflorescences and fruits (Pate 1975, Shelp 1987, 1988). In experiment one, plant-B status and external-B supply did not markedly influence the specific allocation to broccoli florets of B acquired during inflorescence development; this newly acquired B accounted for 68 to 99% of the floret B at 14 DAIE, and previously acquired B the remainder. The relative partitioning of the newly acquired B to florets was lowest in plants with luxury-B status. Forty-four to 79% of the newly acquired B in the plant was recovered in florets.

Fig. 5. Relative partitioning (% of total B enrichment) during broccoli-inflorescence development of B acquired in the 10-day period just before inflorescence emergence (upper panel), and during lupin primary-inflorescence development of B acquired in the 15-day period just before inflorescence emergence (lower panel). With the exception of the 10- or 15-day periods when broccoli and lupin plants were supplied with enriched ^{10}B at 0.5 and 0.3 mg l^{-1} respectively, they received enriched ^{11}B at the same concentration. The key indicates the various strata. Data represent the mean ± SE (n = 5); where the SE is not evident, it is within the symbol.



For lupin, at 7 and 21 DAIE, respectively, 55 to 100% and 9 to 70% of the B in reproductive structures was acquired after inflorescence emergence. The relative partitioning of this newly acquired B was not influenced by plant-B status, and only 4 to 10% of the newly acquired B in the plant was recovered in the reproductive structures. In experiment two, additional evidence was provided for the retranslocation of previously acquired B by broccoli plants receiving sufficient B, with 8% being recovered in florets at 15 DAIE. The degree of retranslocation of previously acquired B from source leaves in lupin was apparently higher than in broccoli, with 21% being recovered in reproductive structures at 30 DAIE. Thus, during the reproductive growth of broccoli, a plant which has a single inflorescence, the florets were fed preferentially with newly acquired B. In lupin, newly acquired B appeared to be somewhat less important; however, comparisons on the quantitative importance of newly vs previously acquired B in the two species were complicated by the growth habit and stage of maturity. Most importantly, the occurrence of B retranslocation in both broccoli and lupin was not dependent on the induction of B deficiency, either by continuous B starvation or transfer to a deficient-B supply after a period of luxury- or sufficient-B supply.

The broccoli cultivar used here (Commander) is relatively less susceptible to B deficiency than three other cultivars examined; when exposed to an interrupted B supply it is the only one in which new growth is supported fully by previously acquired B (Shelp et al. 1992b). Other studies, using plants exposed to an interrupted B supply, showed that previously acquired B satisfies the B requirements of the fruit of peanut and subterranean clover (Campbell et al. 1975), but not the requirements for new vegetative growth in tomato (Oertli 1993). This research, together with the present study, suggest that the B requirements of developing sinks plants may be satisfied by the retranslocation in phloem of both newly and previously acquired B, with the relative importance of the two sources depending, at least in part, on the stage of development and species/cultivar. Given that newly acquired B transiently accumulated in some plant parts, it is tempting to speculate that B, like nitrogen (Pate 1975, Da Silva and Shelp 1990), undergoes direct, as well as indirect xylem-to-phloem transfer. The present study is consistent with earlier work showing that B is continually required in small amounts throughout the life cycle to maintain a healthy plant (Warrington 1923), and provides an explanation for the effectiveness of late-season applications of B to field and horticultural crops (Anonymous 1991).

In summary, we used the boron mass isotope ^{10}B to trace the dynamics of B retranslocation in broccoli and lupin plants during reproductive growth as a function of plant-B status and external-B supply. Newly acquired B was an important source of B for reproductive structures, but the relative importance of newly acquired and previously acquired B appeared to be species dependent. Data on the B concentrations of various plant parts and phloem indicated that the typical decreasing acropetal B gradient in plants is poor support for the translocation of B in xylem only, and are consistent with B being a phloem-mobile element.

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